Fig. 1

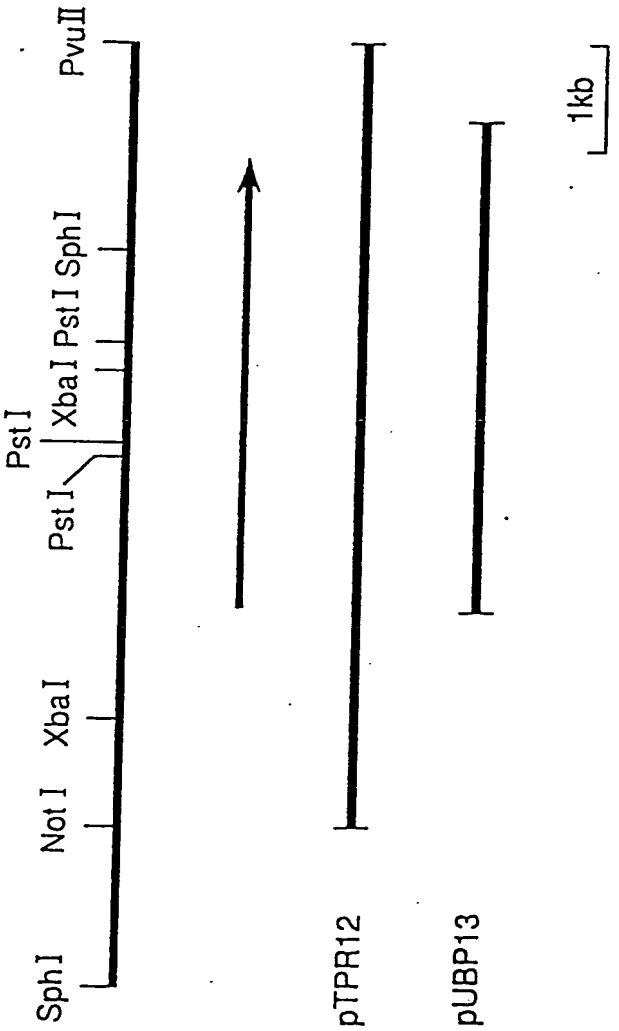


Fig. 2

```
                170                 175                 180
          Asp Gly Ser Gly Val Val Val Ala Val Leu Asp Thr Gly Val
       5'-GAT GGT AGT GGT GTT GTT GTT GCA GTA CTT GAC ACG GGA GTT-3'

PRO-1F    5'-GGW WSD RRT GTT RRH GTH GCD GTD MTY GAC ACB GG-3'
```

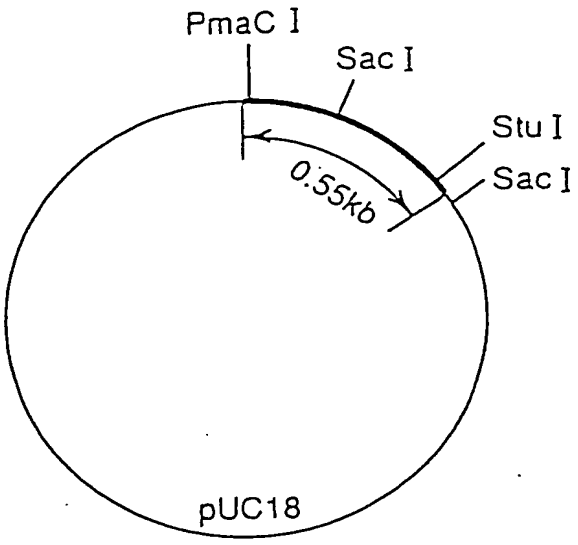
Fig. 3

```
                365                 370                 375
          His Gly His Gly Thr His Val Ala Gly Thr Val Ala Gly Tyr
       5'-CAC GGT CAC GGA ACT CAC GTA GCT GGA ACT GTT GCT GGT TAC-3'

PRO-2F    5'-KST CAC GGA ACT CAC GTD GCB GGH ACD GTT GC-3'
PRO-2R         3'-GTG CCT TGA GTG CAH CGV CCK TGH CAA CGM CSA-5'
```

Fig. 4

```
                590                 595
          Ser Gly Thr Ser Met Ala Thr Pro His Val Ser Gly Val Val
       5'-TCT GGA ACT TCG ATG GCT ACT CCA CAT GTC AGC GGT GTC GTT-3'

PRO-4R    3'-CCD TGV AGB TAC CGD WGA GGB GTR CAV YSG CCH C-5'
```

Fig. 5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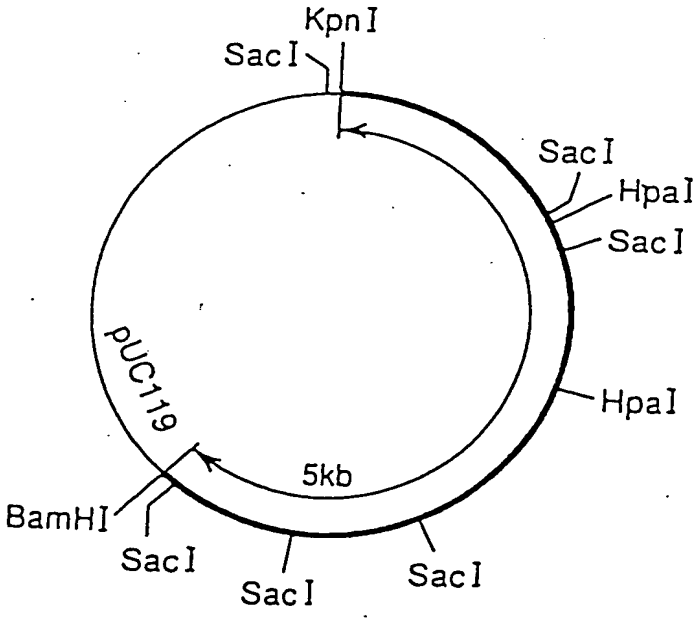
Sac I
Stu I
Sac I
0.55kb
pUC18

Fig. 6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Sac I
Sac I
Hpa I
Sac I
pUC119
Hpa I
5kb
BamHI
Sac I
Sac I
Sac I

Fig. 7

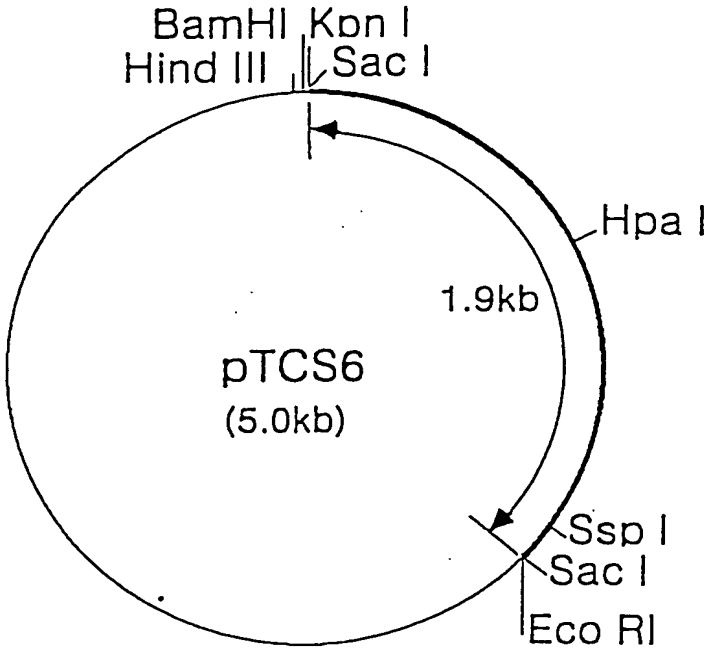
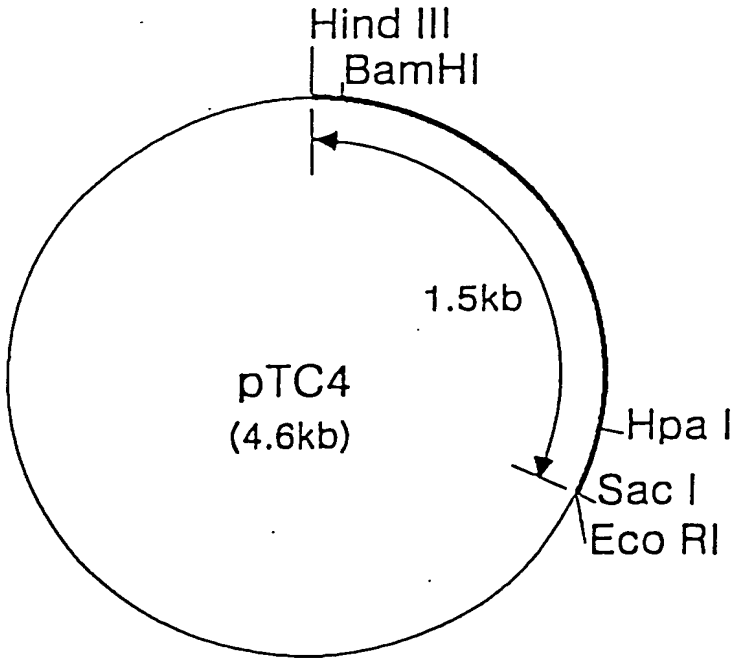

BamHI
Kpn I
Hind III
Sac I
Hpa I
1.9kb
pTCS6
(5.0kb)
Ssp I
Sac I
Eco RI

Fig. 8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BamHI
1.5kb
pTC4
(4.6kb)
Hpa I
Sac I
Eco RI

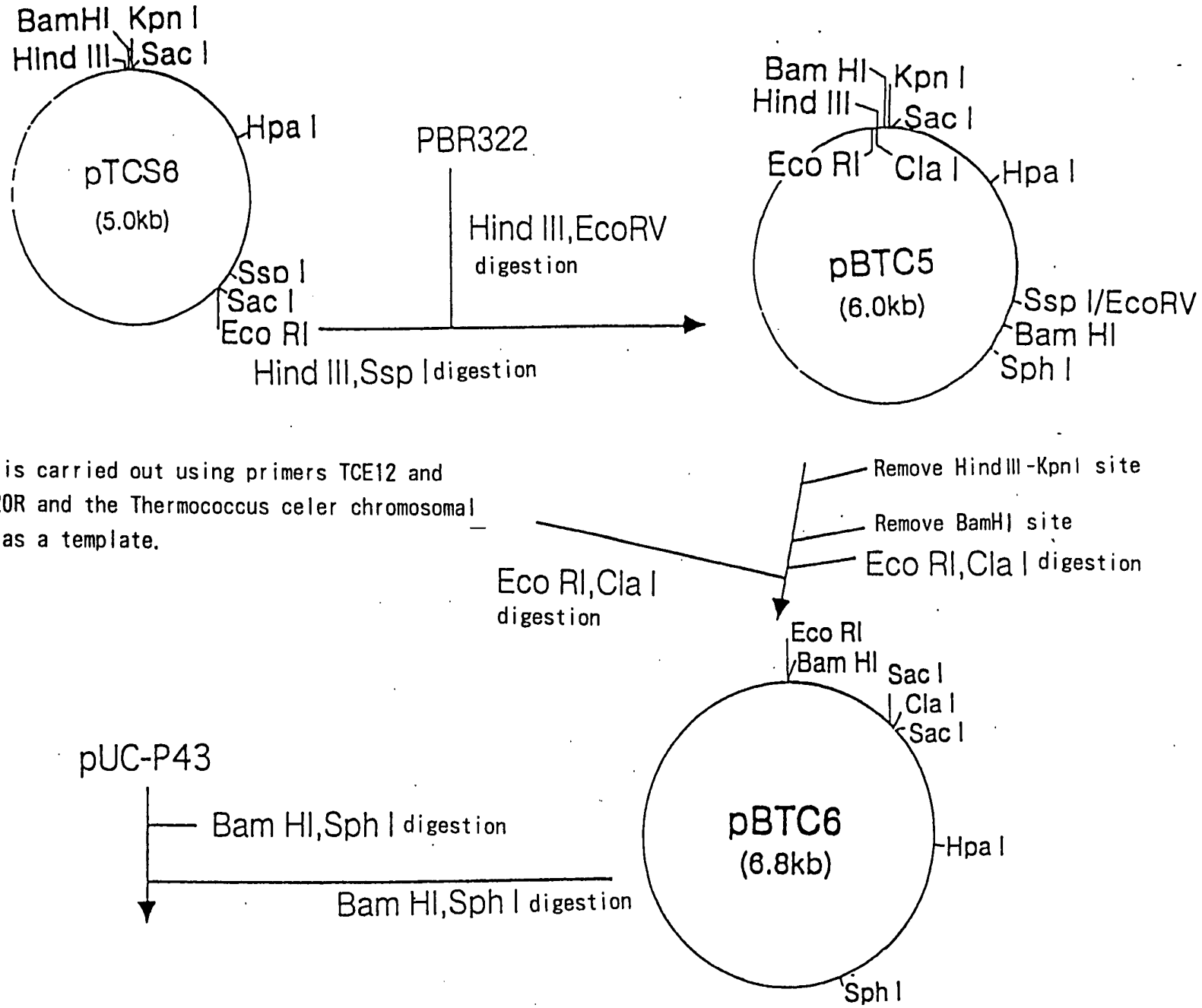

BamHI Kpn I
HInd III Sac I
Hpa I
pTCS6
(5.0kb)
Sso I
Sac I
Eco RI
PBR322
Hind III,EcoRV
digestion
Hind III,Ssp I digestion
Bam HI
Kpn I
Hind III
Sac I
Eco RI
Cla I
Hpa I
pBTC5
(6.0kb)
Ssp I/EcoRV
Bam HI
Sph I
PCR is carried out using primers TCE12 and TCE20R and the Thermococcus celer chromosomal DNA as a template.
Remove HindIII-KpnI site
Remove BamHI site
Eco RI,Cla I digestion
Eco RI,Cla I
digestion
Eco RI
Bam HI
Sac I
Cla I
Sac I
pUC-P43
Bam HI,Sph I digestion
Bam HI,Sph I digestion
pBTC6
(6.8kb)
Hpa I
Sph I

Fig. 9

Fig. 9(Cont'd)

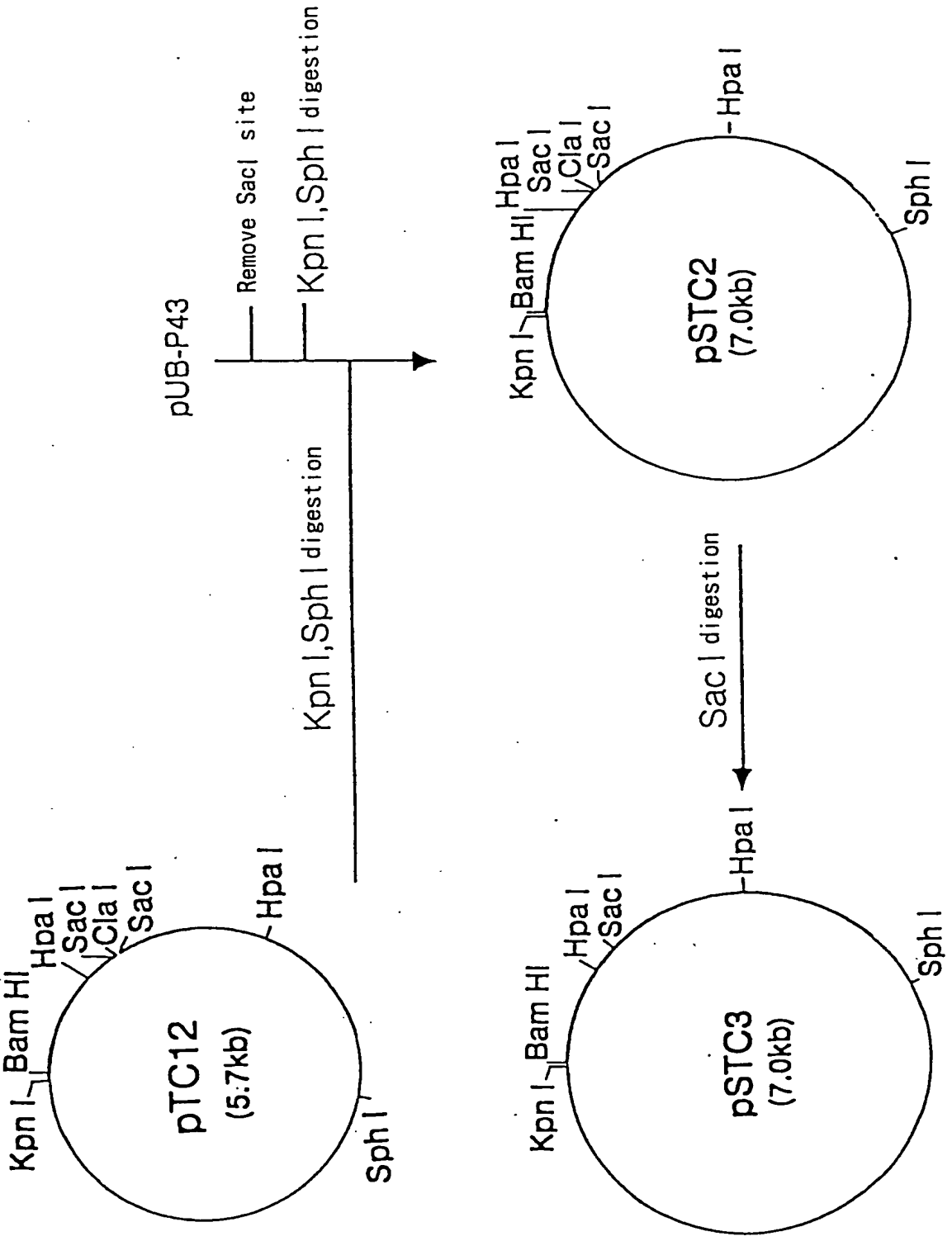

pUB-P43
Remove SacI site
Kpn I,Sph I digestion
Kpn I,Sph I digestion
pTC12
(5.7kb)
Kpn I
Bam HI
Hpa I
Sac I
Cla I
Sac I
Hpa I
Sph I
pSTC2
(7.0kb)
Kpn I
Bam HI
Hpa I
Sac I
Cla I
Sac I
Hpa I
Sph I
Sac I digestion
pSTC3
(7.0kb)
Kpn I
Bam HI
Hpa I
Sac I
Hpa I
Sph I

Fig. 10

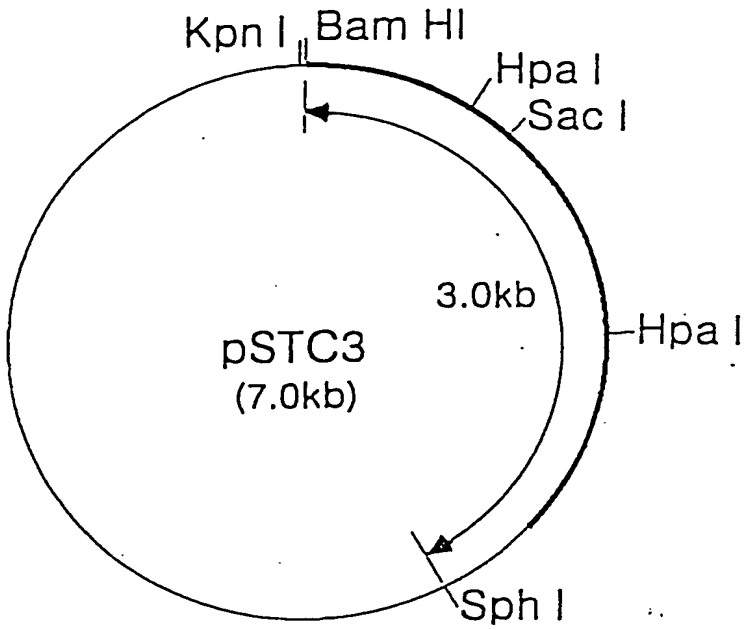


Fig. 11

```
                      10         20         30         40         50
PFUL         MNKKGLTVLF IAIMLLSVVP VHFVSAETPP VSSENSTTSI LPNQQVVTKE
TCES                      MKRLGAVV LALVLVGLLA GTALAAPVKP VVRNNAVQQK
SUBTILISIN                                               MRGKKVWISL

                      60         70         80         90        100
PFUL         VSQAALNAIM KGQPNMVLII KTKEGKLEEA KTELEKLGAE ILDENRVLNM
TCES         NYGLLTPGLF KKVQRMNWNQ EVDTVIMFGS YGDRDRAVKV LRLMGAQVKY
SUBTILISIN   LFALALIFTM AFGSTSSAQA AGKSNGEKKY IVGFKQTMST MSAAKKKDVI

                     110        120        130        140        150
PFUL         LLVKIKPEKV KELNYISSLE KAWLNREVKL SPPIVEKDVK TKEPSLEPKM
TCES         SYKIIPAVAV KIKARDLLLI AGMIDTGYFG NTRVSGIKFI QEDYKVQVDD
SUBTILISIN   SEKGGKVQKQ FKYVDAASAT LNEKAVKELK KDPSVAYVEE DHVAHAYAQS

                     160        170        180        190        200
PFUL         YNSTWVINAL QFIQEFGYDG SGVVVAVLDT GVDPNHPFLS ITPDGRRKII
TCES         ATSVSQIGAD TVWNSLGYDG SGVVVAIVDT GIDANHPDLK GKVIGWYDAV
SUBTILISIN   VPYGVSQIKA PALHSQGYTG SNVKVAVIDS GIDSSHPDLK VAGGASMVPS

                     210        220        230        240        250
PFUL         EWKDFTDEGF VDTSFSFSKV VNGTLIINTT FQVASGLTLN ESTGLMEYVV
TCES         NGRSTPYDDQ ---------- ---------- ---------- ----------
SUBTILISIN   ETNPFQDNN- ---------- ---------- ---------- ----------

                     260        270        280        290        300
PFUL         KTVYYVSNVTI GNITSANGIY HFGLLPERYF DLNFDGDQED FYPVLLVNST
TCES         ---------- ---------- ---------- ---------- ----------
SUBTILISIN   ---------- ---------- ---------- ---------- ----------
```

Fig. 11 (Cont'd)

```
                 310        320        330        340        350
PFUL        GNGYDIAYVD TDLDYDFTDE VPLGQYNVTY DVAVFSYYYG PLNYVLAEID
TCES        ---------- ---------- ---------- ---------- ----------
SUBTILISIN  ---------- ---------- ---------- ---------- ----------

                 360        370        380        390        400
PFUL        PNGEYAVFGW DGHGHGTHVA GTVAGYDSNN DAWDWLSMYS GEWEVFSRLY
TCES        ---------- ---GHGTHVA GIVAGTGSVN SQ-------- ----------
SUBTILISIN  ---------- ---SHGTHVA GTVAA--LNN SI-------- ----------

                 410        420        430        440        450
PFUL        GWDYTNVTTD TVQGVAPGAQ IMAIRVLRSD GRGSMWDIIE GMTYAATHGA
TCES        ---------- -YIGVAPGAK LVGVKVLGAD GSGSVSTIIA GVDWVVQNKD
SUBTILISIN  ---------- GVLGVAPSAS LYAVKVLGAD GSGQYSWIIN GIEWAIANNM

                 460        470        480        490        500
PFUL        ----DVISMS LGGNAPYLDG TDPESVAVDE LTEKYGVVFV IAAGNEGPGI
TCES        KYGIRVINLS LGSSQSS-DG TDSLSQAVNN AWDA-GIVVC VAAGNSGPNT
SUBTILISIN  ----DVINMS LGGP----SG SAALKAAVDK AVAS-GVVVV AAAGNEGTSG

                 510        520        530        540        550
PFUL        N--IVGSPGV ATKAITVGAA AVPINVGVYV SQALGYPDYY GFYYFPAYTN
TCES        Y--TVGSPAA ASKVITVGAV DSNDN----- ---------- ----------
SUBTILISIN  SSSTVGYPGK YPSVIAVGAV DSSNQ----- ---------- ----------

                 560        570        580        590        600
PFUL        VRIAFFSSRG PRIDGEIKPN VVAPGYGIYS SLPMWIGGAD F-------MS
TCES        --IASFSSRG PTADGRLKPE VVAPGVDIIA PRASGTSMGT PINDYYTKAS
SUBTILISIN  --RASFSSVG PELD------ VMAPGVSIQS TLPGNKYGA- --------YN
```

Fig. 12

```
                   610        620        630        640        650
PFUL        GTSMATPHVS GVVALLISGA KAEGIYYNPD IIKKVLESGA TWLEGDPYTG
TCES        GTSMATPHVS GVAALILQAH PSWTPDKVKT ----ALIETA DIVAPKEIAD
SUBTILISIN  GTSMASPHVA GAAALILSKH PNWTNTQVRS ----SLENTT TKL-GDS---

                   660        670        680        690        700
PFUL        QKYTELDQGH GLVNVTKSWE ILKAINGTTL PIVDHWADKS YSDFAEYLGV
TCES        -----IAYGA GRVNVYKAIK YDDYAKLTFT GSVADKGSAT HTFDVSGATF
SUBTILISIN  -----FYYGK GLINVQAAAQ *

                   710        720        730        740        750
PFUL        DVIRGLYARN SIPDIVEWHI KYVGDTEYRT FEIYATEPWI KPFVSGSVIL
TCES        VTATLYWDTG SSDIDLYLYD PNGNEVDYSY TAYYGFEKVG YYNPTAGTWT

                   760        770        780        790        800
PFUL        ENNTEFVLRV KYDVEGLEPG LYVGRIIIDD PTTPVIEDEI LNTIVIPEKF
TCES        VKVVSYKGAA NYQVDVVSDG SLSQSGGGNP NPNPNPNPTP TTDTQTFTGS

                   810        820        830        840        850
PFUL        TPENNYTLTW YDINGPEMVT HHFFTVPEGV DVLYAMTTYW DYGLYRPDGM
TCES        VNDYWDTSDT FTMNVNSGAT KITGDLTFDT SYNDLDLYLY DPNGNLVDRS

                   860        870        880        890        900
PFUL        FVFPYQLDYL PAAVSNPMPG NWELVWTGFN FAPLYESGFL VRIYGVEITP
TCES        TSSNSYEHVE YANPAPGTWT FLVYAYRTYG WADYQLKAVV YYG*

                   910        920        930        940        950
PFUL        SVWYINRTYL DTNTEFSIEF NITNIYAPIN ATLIPIGLGT YNASVESVGD
```

Fig. 12(Cont'd)

	960	970	980	990	1000
PFUL	GEFFIKGIEV	PEGTAELKIR	IGNPSVPNSD	LDLYLYDSKG	NLVALDGNPT
	1010	1020	1030	1040	1050
PFUL	AEEEVVVEYP	KPGVYSIVVH	GYSVRDENGN	PTTTTFDLVV	QMTLDNGNIK
	1060	1070	1080	1090	1100
PFUL	LDKDSIILGS	NESVVVTANI	TIDRDHPTGV	YSGIIEIRDN	EVYQDTNTSI
	1110	1120	1130	1140	1150
PFUL	AKIPITLVID	KADFAVGLTP	AEGVLGEARN	YTLIVKHALT	LEPVPNATVI
	1160	1170	1180	1190	1200
PFUL	IGNYTYLTDE	NGTVTFTYAP	TKLGSDEITV	IVKKENFNTL	EKTFQITVSE
	1210	1220	1230	1240	1250
PFUL	PEITEEDINE	PKLAMSSPEA	NATIVSVEME	SEGGVKKTVT	VEITINGTAN
	1260	1270	1280	1290	1300
PFUL	ETATIVVPVP	KKAENIEVSG	DHVISYSIEE	GEYAKYVIIT	VKFASPVTVT
	1310	1320	1330	1340	1350
PFUL	VTYTIYAGPR	VSILTLNFLG	YSWYRLYSQK	FDELYQKALE	LGVDNETLAL
	1360	1370	1380	1390	1400
PFUL	ALSYHEKAKE	YYEKALELSE	GNIIQYLGDI	RLLPPLRQAY	INEMKAVKIL
	1410				
PFUL	EKAIEELEGE	E*			

EP0420850A2

Fig. 13

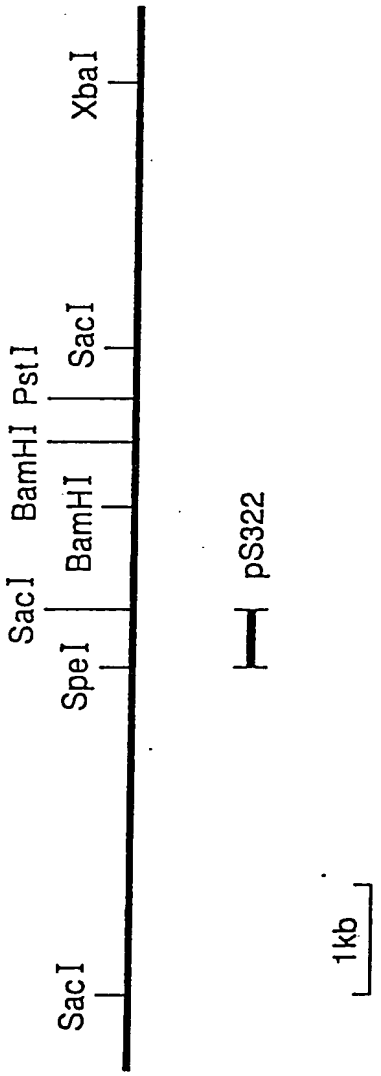
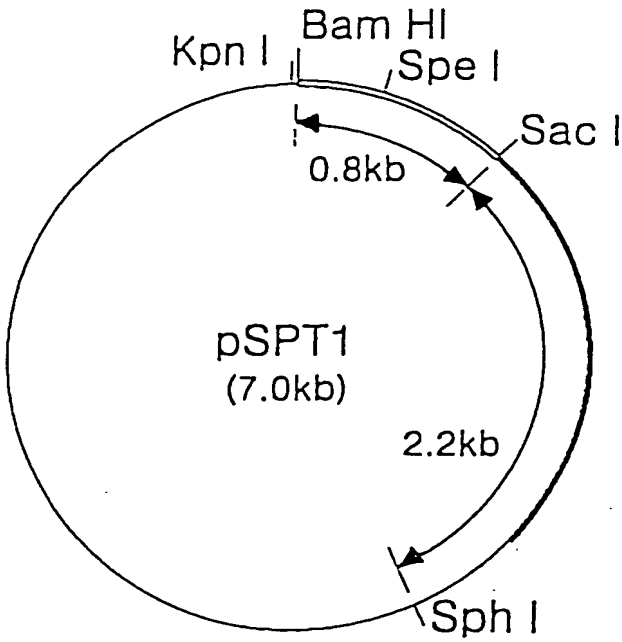


Fig. 14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Kpn I
Spe I
Sac I
0.8kb
pSPT1
(7.0kb)
2.2kb
Sph I

Fig. 15

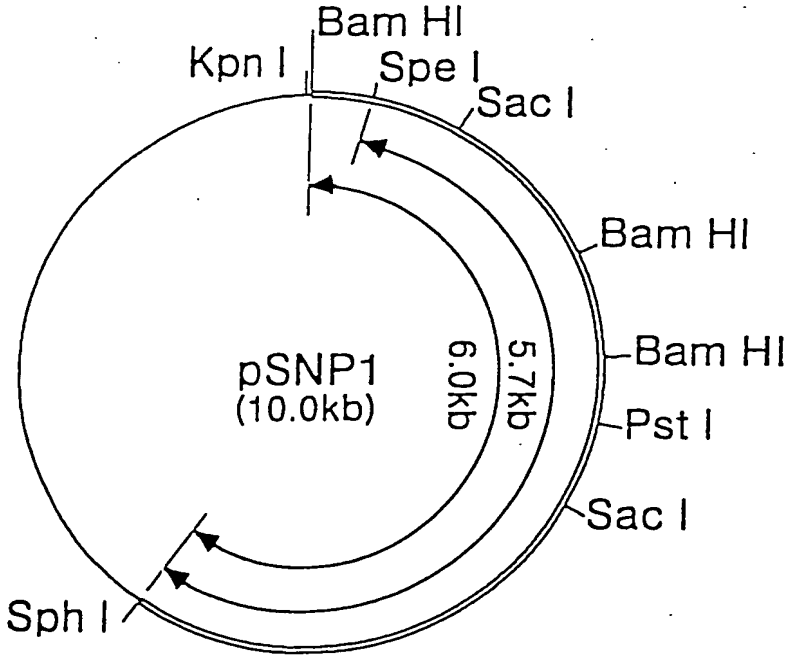

Bam HI
Kpn I
Spe I
Sac I
Bam HI
Bam HI
Pst I
Sac I
pSNP1
(10.0kb)
6.0kb
5.7kb
Sph I

Fig. 16

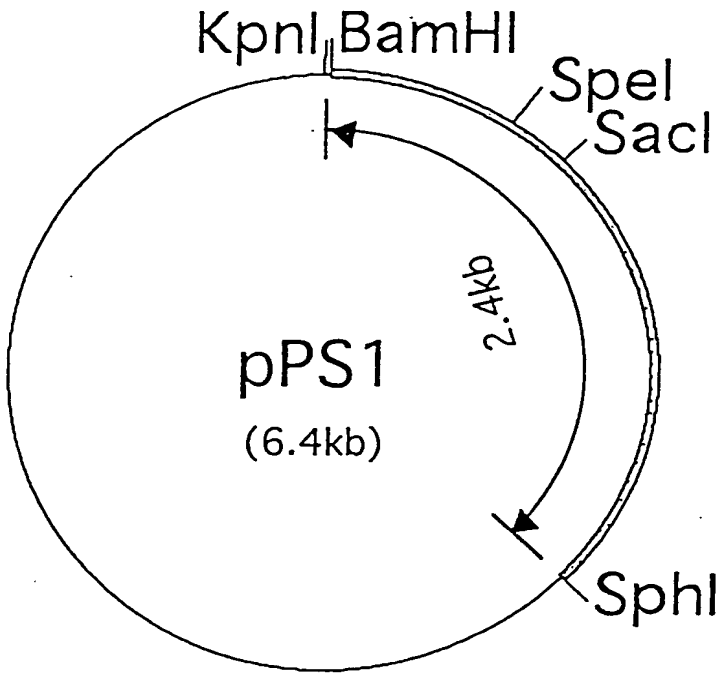
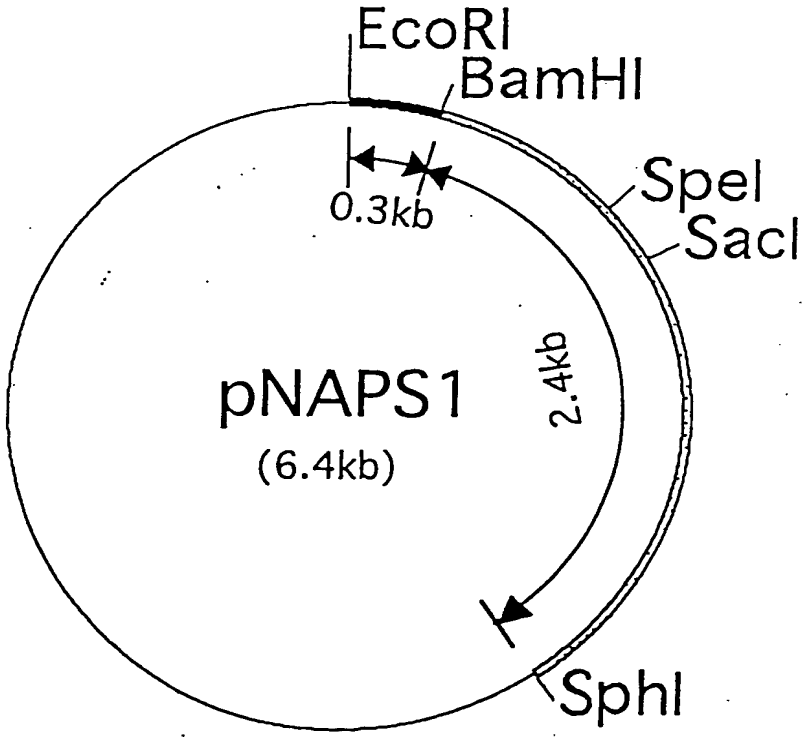

KpnI
BamHI
SpeI
SacI
2.4kb
pPS1
(6.4kb)
SphI

Fig. 17


EcoRI
BamHI
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SpeI
SacI
2.4kb
pNAPS1
(6.4kb)
SphI

Fig. 18

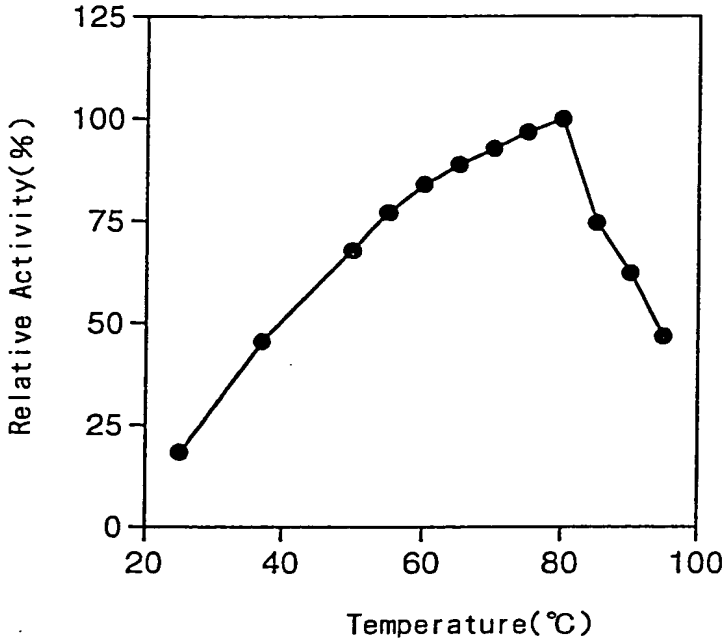

125
100
75
50
25
0
Relative Activity(%)
20
40
60
80
100
Temperature(℃)

Fig. 19

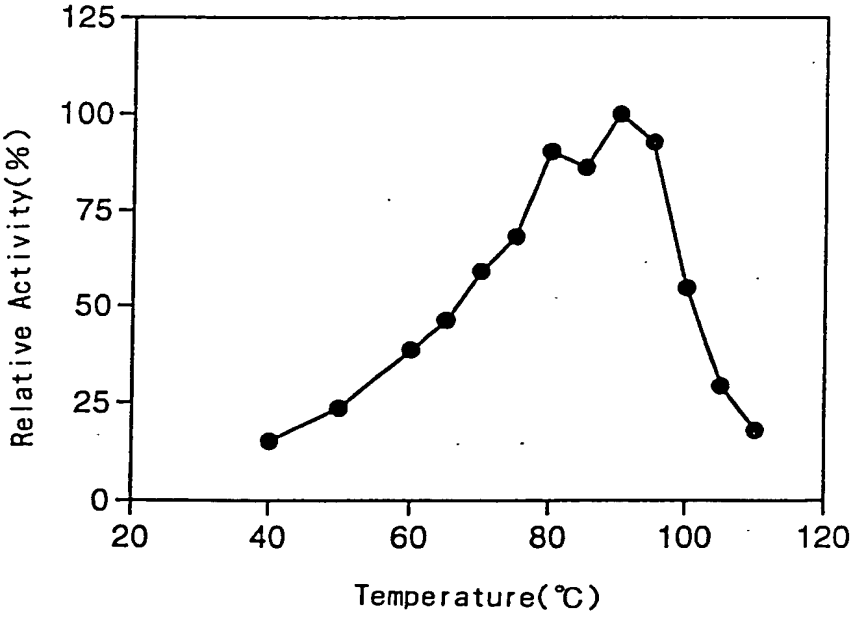
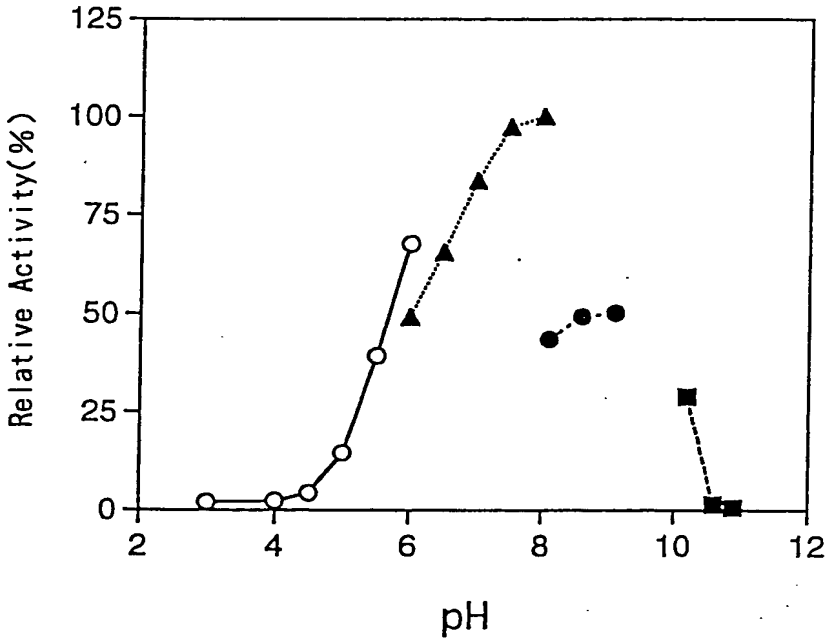

125
100
75
50
25
0
Relative Activity(%)
20
40
60
80
100
120
Temperature(℃)

Fig. 20



—○— Sodium acetate buffer

·····▲····· Sodium phosphate buffer

----●---- Sodium borate buffer

----■---- Sodium phosphate-sodium hydroxide buffer

Fig. 21

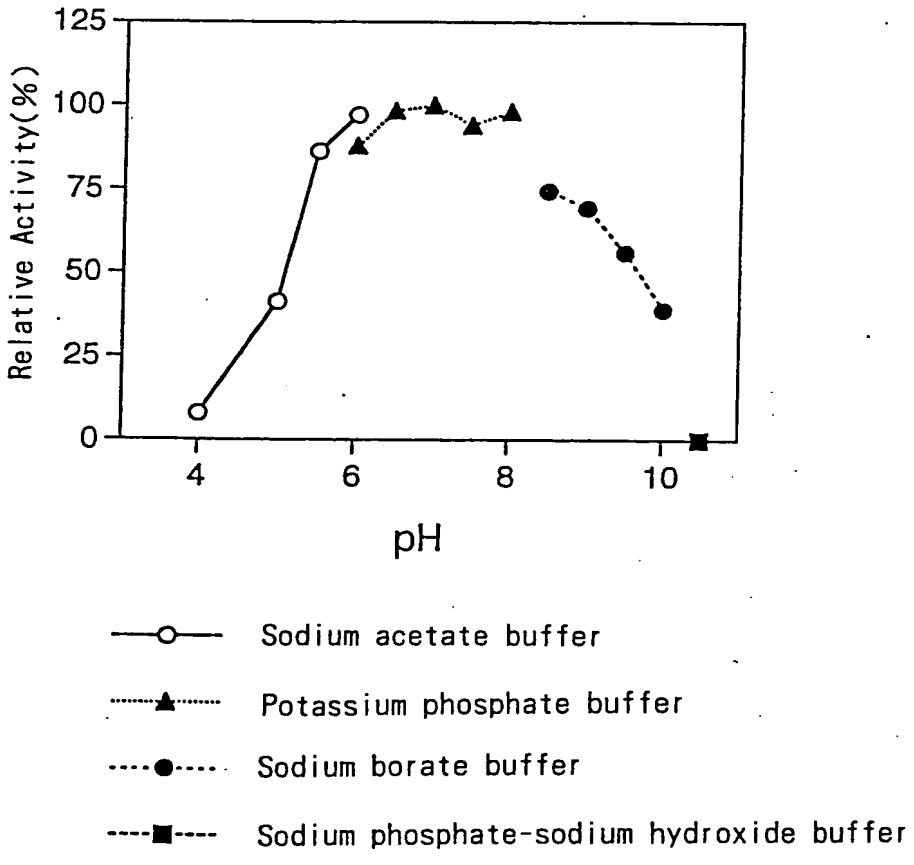

125
100
75
50
25
0
Relative Activity(%)
4
6
8
10
pH
Sodium acetate buffer
Potassium phosphate buffer
Sodium borate buffer
Sodium phosphate-sodium hydroxide buffer

Fig. 22

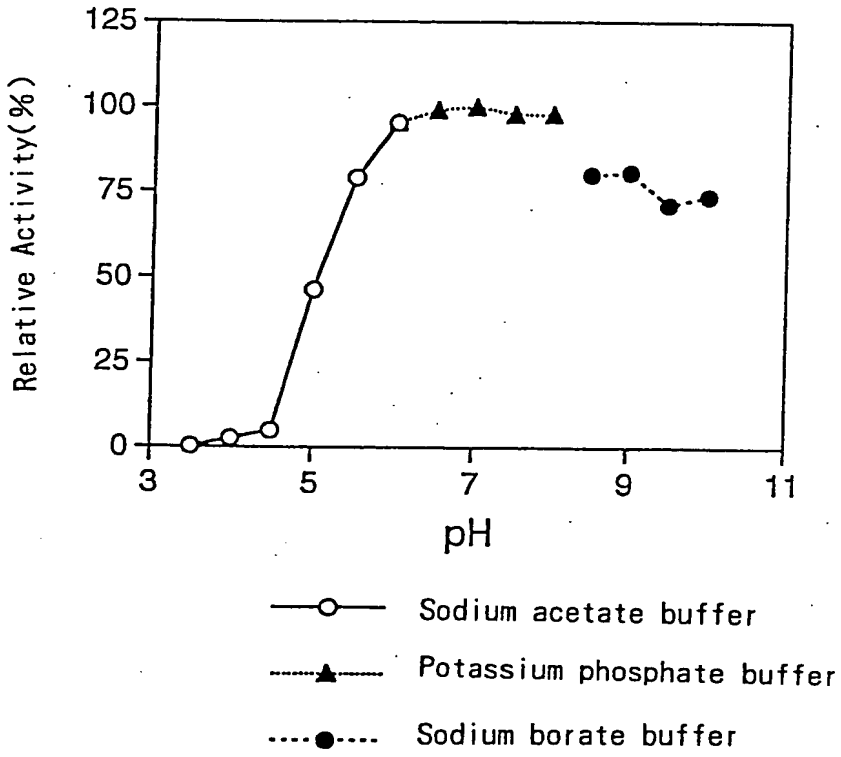

125
100
75
50
25
0
Relative Activity(%)
3
5
7
9
11
pH
Sodium acetate buffer
Potassium phosphate buffer
Sodium borate buffer

Fig. 23

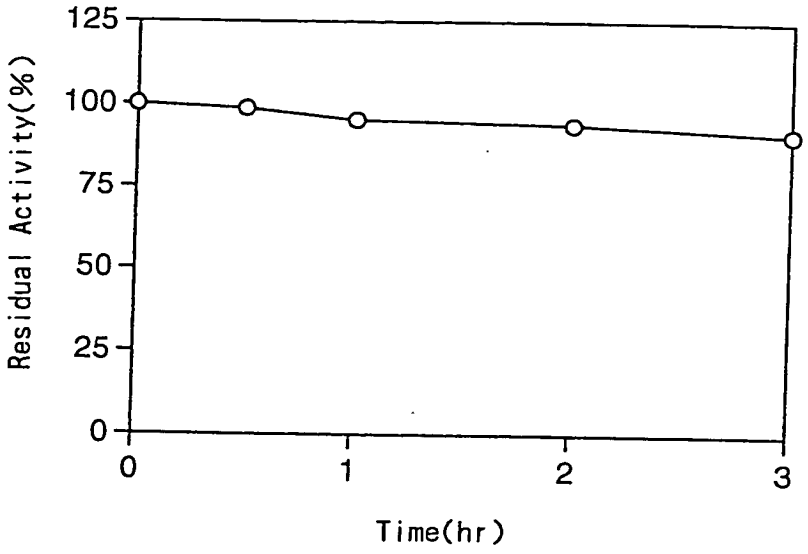

125
100
75
50
25
0
Residual Activity(%)
0
1
2
3
Time(hr)

Fig. 24

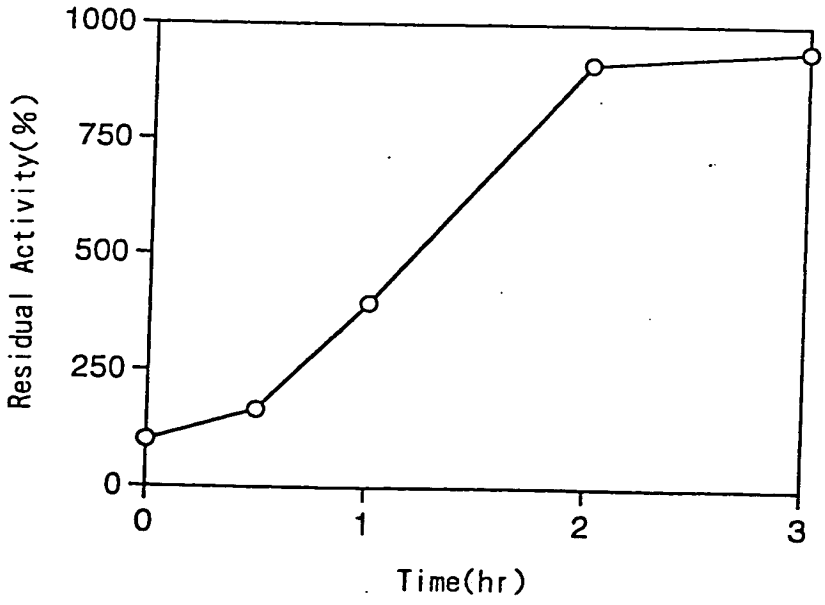

1000
750
500
250
0
Residual Activity(%)
0
1
2
3
Time(hr)

Fig. 25

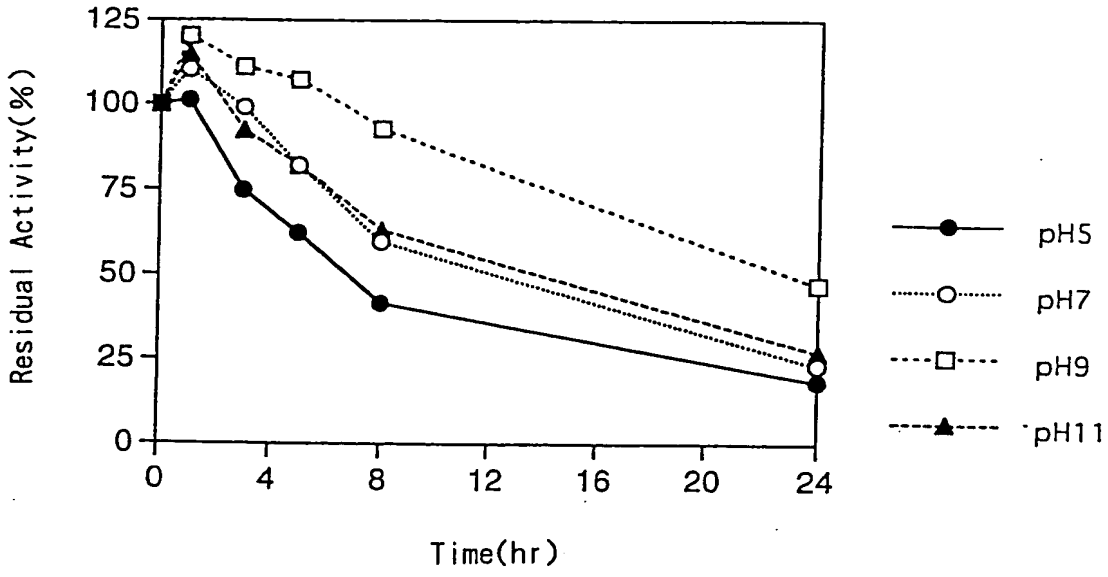

125
100
75
50
25
0
Residual Activity(%)
0
4
8
12
16
20
24
Time(hr)
pH5
pH7
pH9
pH11

Fig. 26

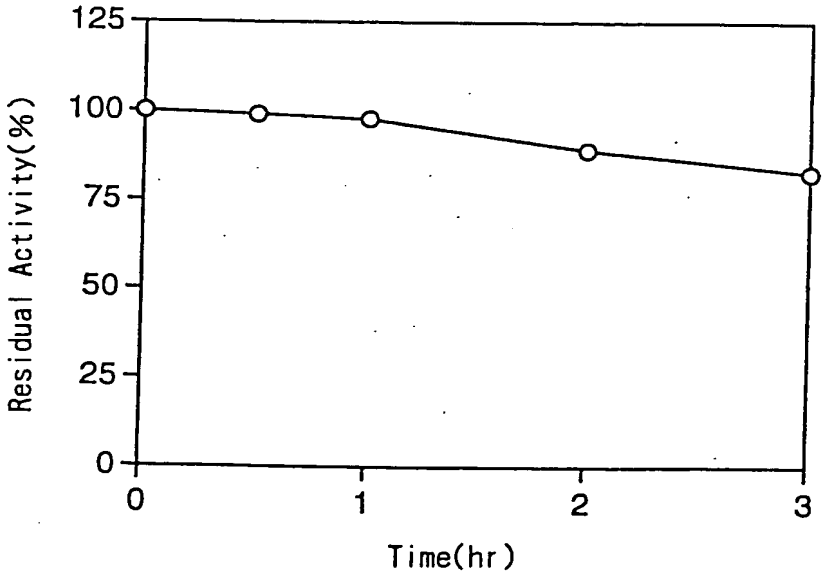

125
100
75
50
25
0
Residual Activity(%)
0
1
2
3
Time(hr)

Fig. 27

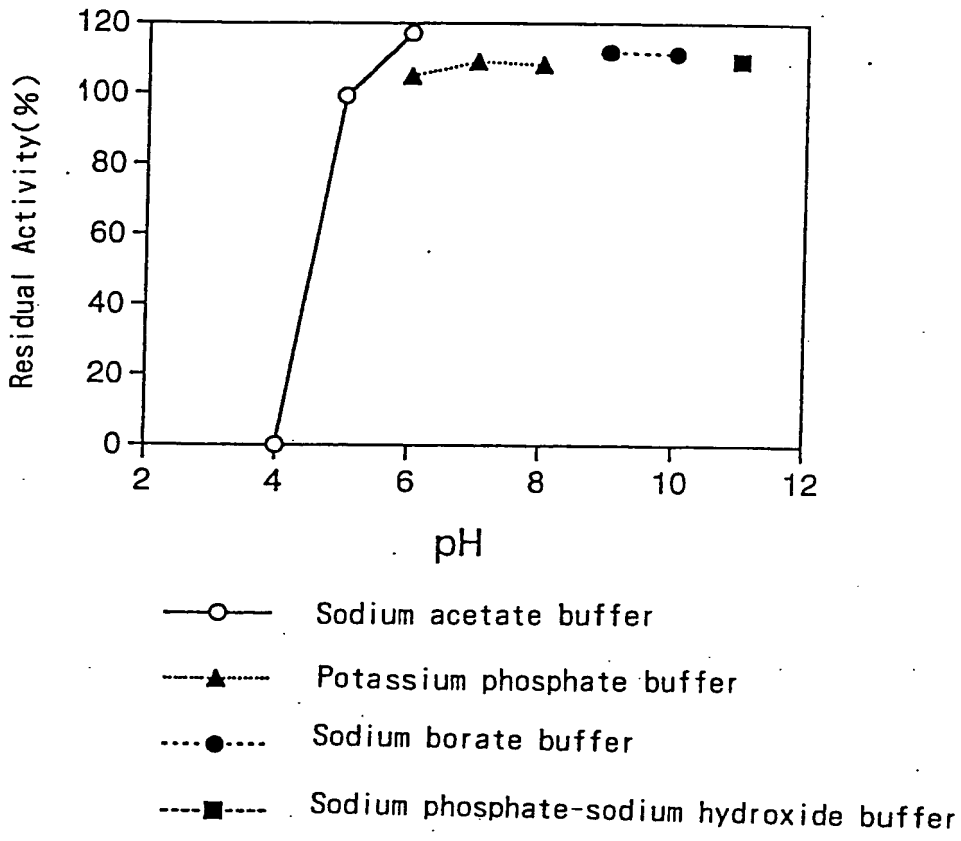

Residual Activity(%)
120
100
80
60
40
20
0
2
4
6
8
10
12
pH
Sodium acetate buffer
Potassium phosphate buffer
Sodium borate buffer
Sodium phosphate-sodium hydroxide buffer

Fig. 28

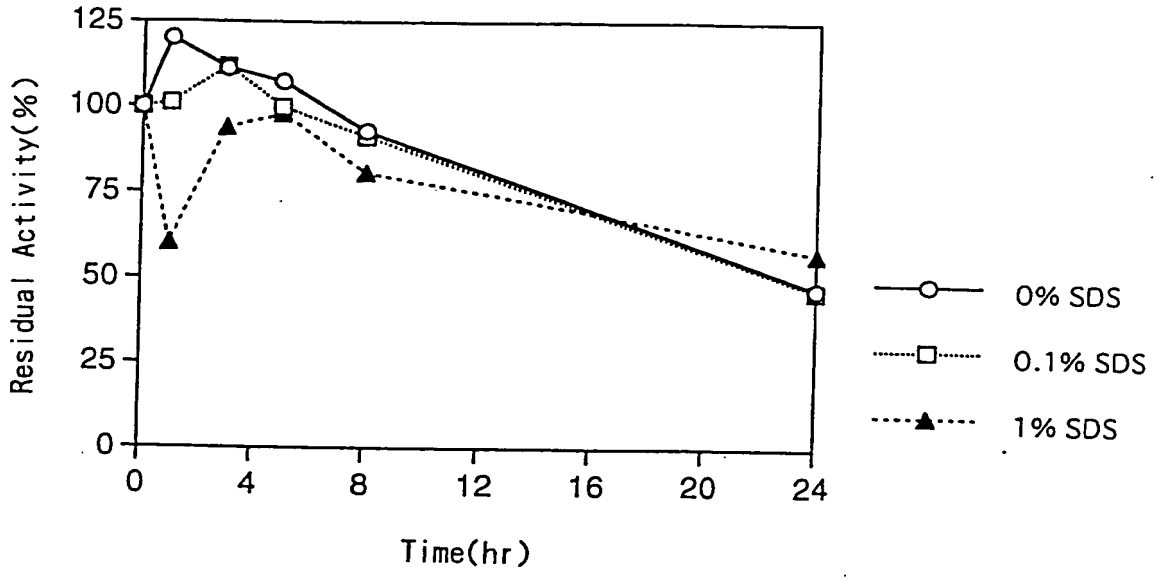

125
100
75
50
25
0
Residual Activity(%)
0
4
8
12
16
20
24
Time(hr)
0% SDS
0.1% SDS
1% SDS

Fig. 29

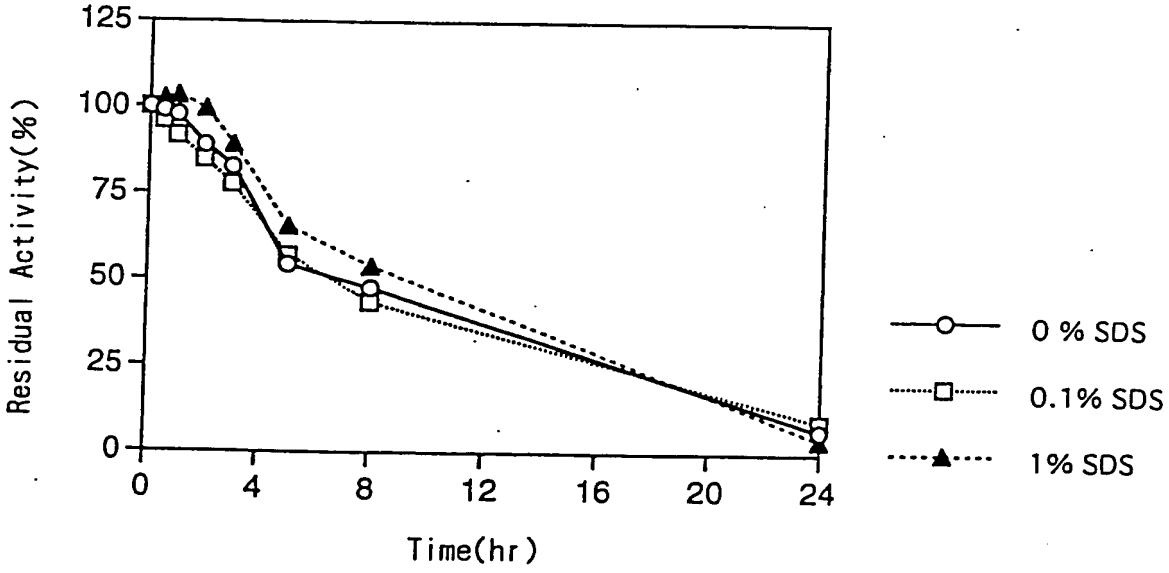

125
100
75
50
25
0
Residual Activity(%)
0
4
8
12
16
20
24
Time(hr)
0 % SDS
0.1% SDS
1% SDS

Fig. 30

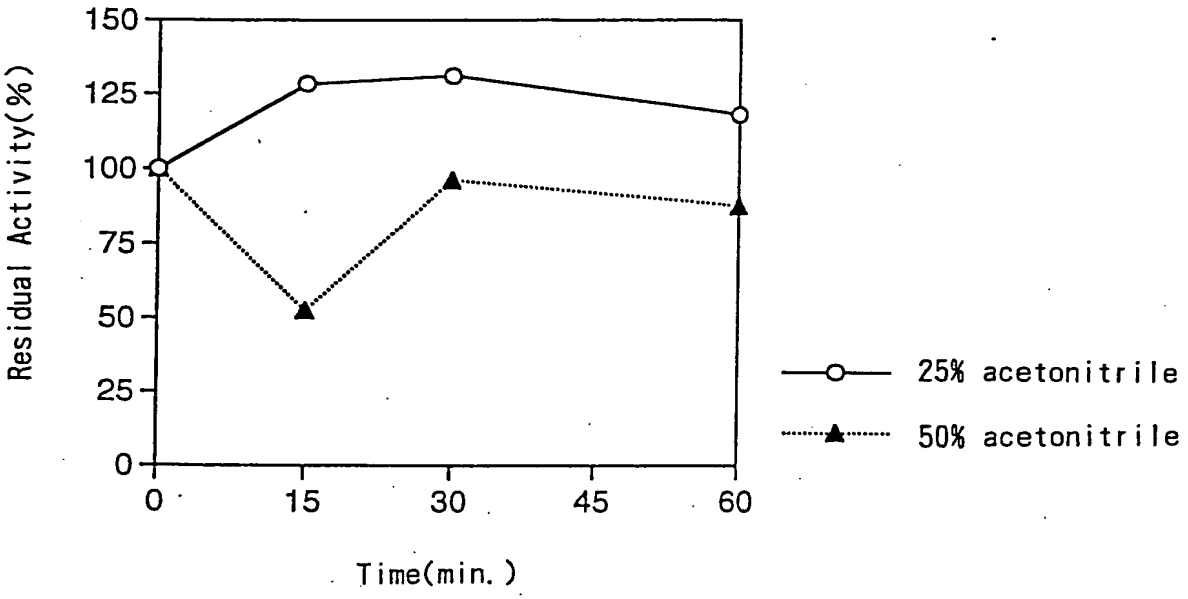

150
125
100
75
50
25
0
Residual Activity(%)
0
15
30
45
60
Time(min.)
25% acetonitrile
50% acetonitrile

Fig. 31

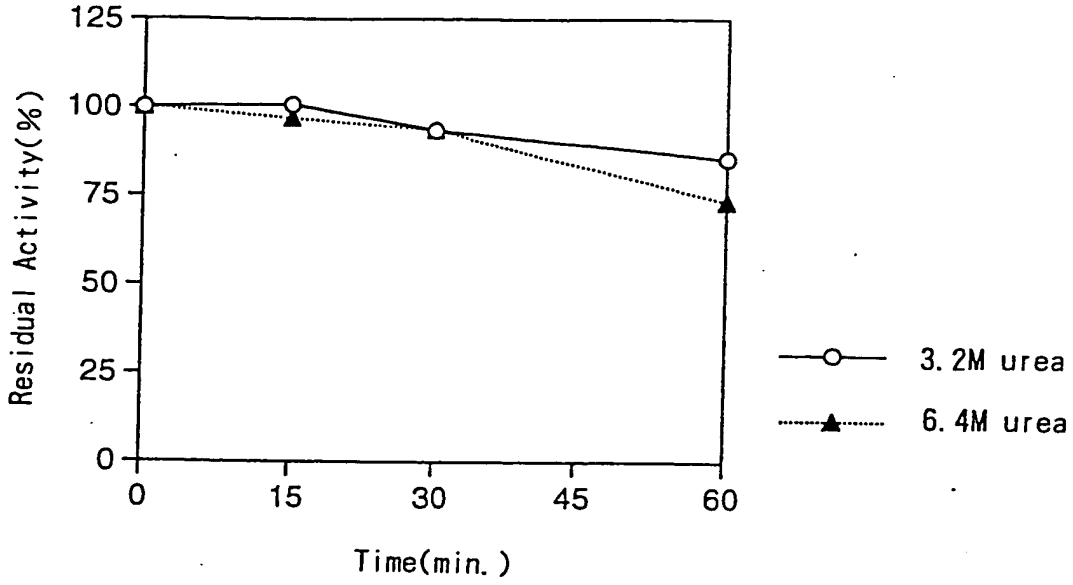
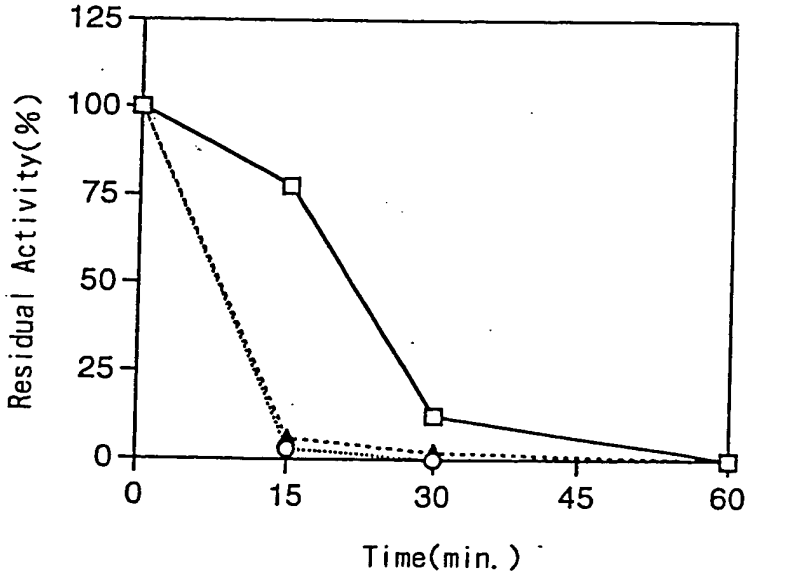


Fig. 32



1M guanidine hydrochloride

3.2M guanidine hydrochloride

6.4M guanidine hydrochloride